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Activation of Phosphatidylinositol 3-Kinase in Cells Expressing *abl* Oncogene Variants

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A phosphoinositide kinase specific for the D-3 position of the inositol ring, phosphatidylinositol (PI) 3-kinase, associates with activated receptors for platelet-derived growth factor, insulin, and colony-stimulating factor 1, with products of the oncogenes *src*, *fms*, *yes*, *crk*, and with polyomavirus middle T antigen. Efficient fibroblast transformation by proteins of the *abl* and *src* oncogene families requires activation of their protein-tyrosine kinase activity and membrane association via an amino-terminal myristoylation. We have demonstrated that the PI 3-kinase directly associates with autophosphorylated, activated protein-tyrosine kinase variants of the *abl* protein. In vivo, this association leads to accumulation of the highly phosphorylated products of PI 3-kinase, PI-3,4-bisphosphate and PI-3,4,5-trisphosphate, only in myristoylated, transforming *abl* protein variants. Myristoylation thus appears to be required to recruit PI 3-kinase activity to the plasma membrane for in vivo activation and correlates with the mitogenicity of the *abl* protein variants.

The protein-tyrosine kinase activities of several growth factor receptors and oncogene products have been implicated in mitogenesis and transformation. Kinase-inactive mutants of the platelet-derived growth factor (PDGF) receptor and other protein-tyrosine kinase-containing receptors fail to stimulate cell division in response to their respective ligands (5, 6, 10, 14, 15), and kinase-defective v-*abl*, v-*fps*, and v-*src* oncogene mutants do not transform cells, suggesting that protein-tyrosine phosphorylation regulates critical events in the control of cell proliferation (2, 32, 35, 42). Whereas the growth factor receptors are transmembrane proteins with ligand-binding properties, cellular proteins of the *abl* and *src* oncogene families associate with the inner surface of the plasma membrane via amino-terminal myristoylation. *src* and *abl* protein mutants which cannot be myristoylated no longer associate with the plasma membrane and are defective in cell transformation (7, 13, 25, 33). These observations suggest either that the critical substrates of the tyrosine kinases are at the plasma membrane or that the substrates must be recruited to the plasma membrane following activation of the protein-tyrosine kinases.

Several enzymes that participate in signal transduction in association with protein-tyrosine kinases have recently been described. Phosphatidylinositol (PI)-specific phospholipase C gamma is phosphorylated on tyrosine and associates with the PDGF and epidermal growth factor receptors following ligand binding (20, 24, 27, 41). Activation of phospholipase C is temporally linked to activation of several growth factor receptors and with cell transformation induced by oncogenes. *ras*-GAP (ras GTPase-activating protein) and the proto-oncogene c-*raf* also form a complex with the PDGF receptor following ligand binding (21, 29, 30, 31). A phosphoinositide kinase that phosphorylates the D-3 position of the inositol ring (PI 3-kinase), associates with the receptors for PDGF, insulin, epidermal growth factor, and colony-stimulating factor 1 (CSF-1), with products of the oncogenes *src*, *fms*, *yes*, *crk*, and with polyomavirus middle T antigen (mT) (11, 12, 19, 34, 38, 40, 44). PI 3-kinase has been purified to homogeneity from rat liver and contains 110- and 85-kDa subunits (4). The 85-kDa subunit of PI 3-kinase is found in a complex with the PDGF-R and mT/pp60$^{c\text{-}src}$ and is phosphorylated on serine and tyrosine residues (19). Elevated levels of PI 3-kinase products are found in cells stimulated with growth factors and in transformed cells (1, 36, 40). The role that these phospholipids play in mitogenesis in unknown, but they may act as a novel second messenger system.

We investigated whether PI 3-kinase is activated by normal and transforming *abl* proteins. The v-*abl* protein transforms NIH 3T3 fibroblasts, whereas the BCR/*abl* fusion protein associated with chronic myelogenous leukemia transforms pre-B lymphocytes but not NIH 3T3 fibroblasts (8, 9, 26, 33). A variant of the BCR/*abl* protein which carries the retrovirus *gag* protein sequence at its N terminus (*gag*/BCR/*abl* protein) efficiently transforms NIH 3T3 fibroblasts and resembles the v-*abl* protein in its leukemogenic properties in vivo (9). The *gag*/BCR/*abl* protein associates in part with the plasma membrane (7a.), presumably through the myristoylated N terminus. Naturally occurring c-*abl* protein types are weak protein-tyrosine kinases and do not transform fibroblasts even when expressed at very high levels, whether or not they are myristoylated (16, 39). Structural deletions at the c-*abl* protein N terminus result in activation of protein-tyrosine kinase activity, phosphorylation of the *abl* proteins on Tyr residues in vivo, and transformation (16). In the present study, we report that certain cellular products of PI 3-kinase are elevated only in cells expressing transforming variants of the *abl* protein.

MATERIALS AND METHODS

Cell lines. All cell lines were derived from NIH 3T3 cells and maintained in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% calf serum and antibiotics. The transformed cell line expressing v-*abl* was

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generated by cotransfection of a construct encoding the 160-kDa *gag*/v-*abl* protein (v-*abl* protein) with Moloney helper virus. The generation of transformed cell lines expressing *gag*/BCR/*abl*, the c-*abl* mutant Δ×B, and the nontransformed cell lines expressing high levels of c-*abl* type IV and p210 (BCR/*abl*) proteins has been described previously (9, 16).

The cell line expressing the *gag*/BCR/*abl* (*gag* p210) fusion protein carries a cloned facsimile of the spontaneously arising p220 *gag*/BCR/*abl* protein which was made by fusing retrovirus *gag* sequences derived from v-*abl* to a BCR sequence by using the 5′ *Hin*cII site in v-*abl* and the *Hin*cII site at position 1194 in the BCR sequence (9, 28). This gene encodes a *gag*/BCR/*abl* protein of 210 kDa, with the first 240 amino acid residues of the *gag* protein replacing the first 241 residues of BCR. When expressed in a retrovirus construct, this protein readily transforms NIH 3T3 fibroblasts. The Δ×B-K290M mutant was made by oligonucleotide-directed mutagenesis of the lysine at position 290 of the tyrosine kinase domain (16a). This residue, which is involved in ATP binding (18), was mutated to methionine. Constructs specifying the Δ×B-K290M mutant were cotransfected at a high molar ratio (10:1) with a plasmid carrying the *neo* gene, which determines resistance to the antibiotic G418. After selection of G418-resistant clones, the clones were screened for high levels of protein expression. The expressed protein is defective in protein-tyrosine kinase function and is unable to transform fibroblasts (16a).

Cell lines were considered transformed on the basis of the following criteria: round and refractile morphology, growth to high density, and ability to form colonies after suspension in semisolid agar medium. Both transformed and nontransformed NIH 3T3 cells expressed *abl* protein variants at high levels relative to endogenous c-*abl* protein.

Association of PI 3-kinase activity with *abl* oncoproteins in vitro. Immunoprecipitable PI 3-kinase was obtained by using monoclonal antibody directed against either phosphotyrosine (anti-P-Tyr antibody was a gift of Brian Drucker) or C-terminal *abl* protein determinants (monoclonal antibody 2421 was a gift of Naomi Rosenberg). NIH 3T3 cells expressing mutant *abl* proteins were grown in DMEM–10% calf serum to confluence for nontransformed cells and to high density for transformed cells. Cells were deprived of serum for 2 to 3 doubling times (36 to 48 h). Cells were lysed in buffer containing 1% Nonidet P-40 as described previously (44). Lysates were incubated with the appropriate antibody for 3 h and collected on protein A-Sepharose beads (Sigma). The beads were washed extensively with lysis buffer, phosphate-buffered saline (PBS), 0.5 M LiCl, and TNE buffers as described previously (44). Immunoprecipitable PI kinase activity was assayed in 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer (pH 7.8)–5 mM $MgCl_2$ in the presence of a 0.2-mg/ml mixture of specific phospholipids with phosphatidylserine as a carrier (the lipids were dried under nitrogen and sonicated for 10 min in 25 mM HEPES [pH 7.8]–1 mM EDTA prior to addition). The reaction was initiated by the addition of 100 μM ATP and 20 μCi of [γ-^{32}P]ATP and stopped after 5 min with an equivalent volume of 0.1 M HCl. The lipids were extracted into methanol-chloroform as described previously (44). A fraction of extracted lipids was separated by thin-layer chromatography in chloroform-methanol-H_2O-concentrated NH_4OH (60:47:11.3:2) and visualized by autoradiography. The remaining phospholipids were subjected to deacylation and fractionated by high-performance liquid chromatography (HPLC) (1, 40). In several experiments, cells were exposed to 10 nmol of PDGF-BB per ml for 10 min prior to lysis to measure cell viability and activation of endogenous PI 3-kinase.

Identification of PI 3-kinase products in vitro and in vivo. A fraction of phospholipids generated in the in vitro PI kinase assay was deacylated in methylamine as described previously (1), suspended in 400 μl of H_2O with appropriate ^{3}H standards, and separated by HPLC on a Partisphere SAX column with an $(NH_4)_2HPO_4$ gradient (0 to 1 M) (40). For in vivo labeling and phospholipid identification, cells were grown to confluence (nontransformed) or high density (transformed) and deprived of serum for 48 h as described above. The cells were labeled with $^{32}P_i$ (0.1 mCi/ml) in phosphate-free minimal essential medium (MEM) for 3 h prior to lysis. Cells were washed with iced MEM and scraped into 0.5 ml of 1 M HCl per 10^7 cells, and the phospholipids were extracted into chloroform-methanol (2:1). Lipid extracts were deacylated and analyzed by HPLC.

RESULTS

Association of PI 3-kinase with *abl* oncoproteins. Cell lysates were analyzed for PI kinase activity by an in vitro immune complex lipid kinase assay. An active PI kinase was detected in anti-P-tyr and anti-*abl* protein immunoprecipitates from fibroblasts expressing the v-*abl*, Δ×B, BCR/*abl*, and *gag*/BCR/*abl* proteins (Fig. 1B, lanes 2 to 11) but not in immunoprecipitates from control NIH 3T3 cells, cells expressing high levels of normal c-*abl* type IV protein (Fig. 1A, lanes 1 and 3, and 1B, lanes 1 and 12), or cells expressing a kinase-activity-defective mutant protein (Δ×B-K290M; data not shown). Similar results were observed when anti-P-Tyr or anti-*abl* protein monoclonal antibodies were used (Fig. 1B). In nontransfected control cells and cells overexpressing the c-*abl* type IV protein, anti-P-Tyr-immunoprecipitable PI kinase activity was detected only following stimulation with PDGF-BB (Fig. 1A, lanes 2 and 4). However, fibroblasts expressing BCR/*abl* protein were not transformed but still had immunoprecipitable PI 3-kinase activity. This activity was detected by using anti-P-Tyr or anti-*abl* protein antibodies (Fig. 1B, lanes 6 and 9). The anti-P-Tyr-immunoprecipitable PI 3-kinase activity increased further to levels comparable to those detected in control NIH 3T3 cells in response to PDGF-BB (Fig. 1B, lanes 6 and 7). A phosphoprotein which comigrated with an 85-kDa phosphoprotein from PDGF-stimulated NIH 3T3 fibroblasts and cells expressing the kinase-inactive K290M mutant protein was phosphorylated in anti-*abl* protein immunoprecipitates from cells transformed by the Δ×B mutant protein (Fig. 2). Similar protein results were obtained with cells expressing *gag*/BCR/*abl* proteins (data not shown).

Identification of the product of immunoprecipitable PI kinase. The products of the lipid kinase activity detected in the anti-*abl* protein immunoprecipitates were deacylated and identified by HPLC. Under the conditions of the assay (i.e., absence of detergents [43]), most of the newly formed phospholipid was PI-3-P (Fig. 3). Similar results were obtained when anti-P-Tyr antibodies were used (data not shown). These results establish that PI 3-kinase directly associates with *abl* protein variants which exhibit activated protein-tyrosine kinase activity, including the nontransforming BCR/*abl* protein.

Accumulation of PI 3-kinase products in intact cells. In quiescent NIH 3T3 fibroblasts, PI-3-P is approximately 4% as abundant as PI-4-P, but the content of PI-3,4-bisphosphate (PI-3,4-P_2) and PI-3,4,5-trisphosphate (PIP_3) is gener-

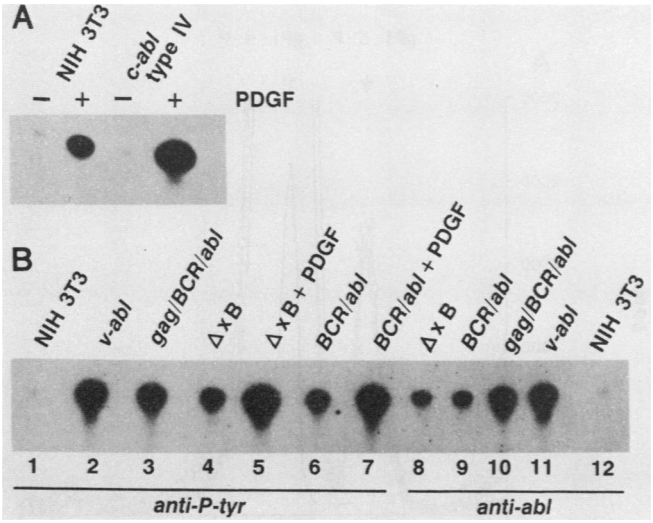

FIG. 1. Association of PI kinase activity with *abl* proteins. (A) PIP formed by immunoprecipitates from control NIH 3T3 cells (lanes 1 and 2) and cells over expressing c-*abl* type IV protein (lanes 3 and 4). Cells were grown to confluency in 100-mm plates and brought to quiescence in DMEM supplemented with 1% bovine serum albumin for the last 48 h of culture. A duplicate plate of each cell line was exposed to 10 ng of PDGF-BB per ml for 10 min (lanes 2 and 4). Cells were lysed in a buffer containing 1% Nonidet P-40, and PI kinase was immunoprecipitated by using monoclonal anti-P-Tyr antibody and protein A-Sepharose beads. PI (0.1 mg/ml) was used as the substrate with 0.1 mg of phosphatidylserine per ml as a carrier with 100 μM ATP and 20 μCi of [γ-^{32}P]ATP. The reaction was terminated by addition of 1 N HCl, and the phospholipids were extracted into chloroform-methanol (2:1), dried under N_2, separated by thin-layer chromatography as described in Materials and Methods, and visualized by autoradiography. The migration position of the newly formed PIP was detected by using nonradioactive PIP which was run in parallel with the samples and visualized by exposure to iodine vapors. (B) PIP formed in immunoprecipitates from whole-cell lysates obtained from control NIH 3T3 cells (lanes 1 and 12) and cells expressing v-*abl* (lanes 2 and 11), *gag*/BCR/*abl* (lanes 3 and 10), Δ×B (lanes 4, 5, and 8), and BCR/*abl* (lanes 6, 7, and 9) proteins. In lanes 5 and 7 cells were exposed to PDGF-BB for 10 min prior to harvest. PI kinase activity was assayed in the immunoprecipitates obtained by using monoclonal anti-P-Tyr (lanes 1 to 7) or monoclonal anti-*abl* protein (lanes 8 to 12) antibodies. The lipid kinase reaction was performed as described above. The products were separated by thin-layer chromatography and visualized by autoradiography.

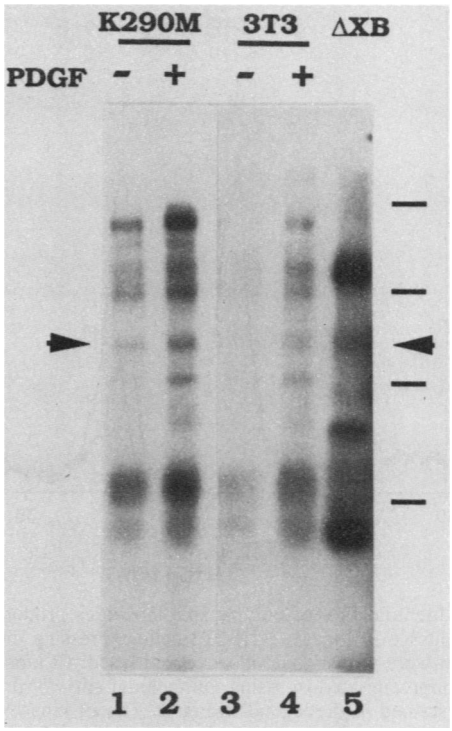

FIG. 2. Detection of an 85-kDa phosphoprotein in immunoprecipitates from cells expressing transforming Δ×B protein. Anti-P-Tyr immunoprecipitates from cells overexpressing the K290M tyrosine kinase-inactive mutant protein (lanes 1 and 2), control NIH 3T3 cells (lanes 3 and 4), and anti-*abl* protein immunoprecipitates from cells expressing transforming Δ×B protein (lanes 5 and 6) were washed as described in Materials and Methods and assayed for protein kinase activity in the presence of 5 mM $MnCl_2$, 100 μM ATP, and [γ-^{32}P]ATP. After 5 min, the reaction was stopped by the addition of sodium dodecylsulfate (SDS)-containing buffer, and the mixture was heated at 70°C for 5 min. Proteins were resolved by SDS–10% polyacrylamide gel electrophoresis and identified by autoradiography. PDGF-BB (10 pg/ml) was added to the cells for 10 min prior to cell lysis as indicated at the top. The lines represent the molecular mass markers at 201, 116, 77, and 46 kDa (Bio-Rad). The 85-kDa phosphoprotein is identified by an arrow.

ally below the detection limit (as judged by incorporation of [^{3}H]inositol; <0.5% of PI-4-P [36]). Detectable incorporation of [^{3}H]inositol or $^{32}P_i$ into PI-3,4-P_2 and PIP_3 appeared upon stimulation with PDGF, CSF-1, and insulin (1, 34, 40). Levels of the same lipids are elevated in cells transformed by polyomavirus mT (36). If expression of transforming *abl* proteins activates PI 3-kinase, the steady-state levels of PI 3-kinase products should be at least transiently elevated. Fibroblasts expressing *abl* protein variants were deprived of serum and labeled with $^{32}PO_4^{3-}$. Total lipids were extracted, deacylated, and analyzed by HPLC as described above. Fibroblasts transformed by v-*abl*, Δ×B, and *gag*/BCR/*abl* proteins had detectable levels of PI-3,4-P_2 and PIP_3. Figure 4 shows a representative HPLC profile identifying the PI-3-P, PI-3,4-P_2, and PIP_3 products in cells transformed by *gag*/BCR/*abl* protein in comparison with cells expressing BCR/*abl* protein and control NIH 3T3 cells. The pattern of phospholipids in the transformed cell lines was similar to that found in PDGF-stimulated smooth muscle cells, CSF-1-stimulated fibroblasts which express the human CSF-1 receptor, and polyomavirus-transformed fibroblasts (1, 36, 40). Very low incorporation of ^{32}P (<0.5% of the total) was detected in PI-3,4-P_2 or PIP_3 in control NIH 3T3 cells (Fig. 4B and C and Fig. 5) or in nontransformed cells expressing high levels of the normal c-*abl* type IV protein or Δ×B kinase-deficient mutant protein (Δ×B-K290M) (data not shown). Quiescent cells expressing nontransforming BCR/*abl* protein had low but detectable incorporation of ^{32}P into PI-3,4-P_2, at about one-half of that found in transformed cells, and no detectable label in PIP_3 (Fig. 4B and C and Fig. 5), which increased to levels comparable to those detected in control NIH 3T3 cells in response to PDGF-BB (data not shown).

DISCUSSION

Transforming variants of the *abl* protein exhibit an activated protein-tyrosine kinase activity and are phosphory-

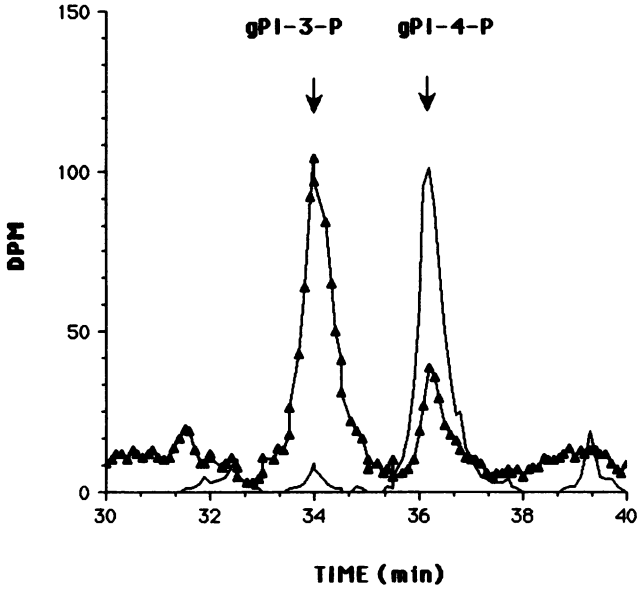

FIG. 3. Identification of polyphosphoinositides produced by immunoprecipitable PI kinase. NIH 3T3 cells expressing the BCR/*abl* oncoprotein were grown to confluence and lysed. PI kinase activity was immunoprecipitated by using monoclonal anti-*abl* protein antibody as described in the legend to Fig. 1. The PI kinase assay was performed with PI as the substrate in the presence of phosphatidylserine as a carrier. The products were extracted into the organic phase, dried under N_2, deacylated, and separated by HPLC as described in Materials and Methods. Closed triangles represent the deacylated product of PIP (gPIP) produced by anti-*abl* protein monoclonal antibody-immunoprecipitable PI kinase from quiescent cells which express the BCR/*abl* protein. The solid line represents the deacylated [^{3}H]PI-4-P standard (NEN) coinjected with the sample.

lated on tyrosine residues in vivo. Normal c-*abl* protein has relatively low but detectable protein-tyrosine kinase activity in vitro. However, c-*abl* protein is not phosphorylated on tyrosine residues in vivo and does not transform NIH 3T3

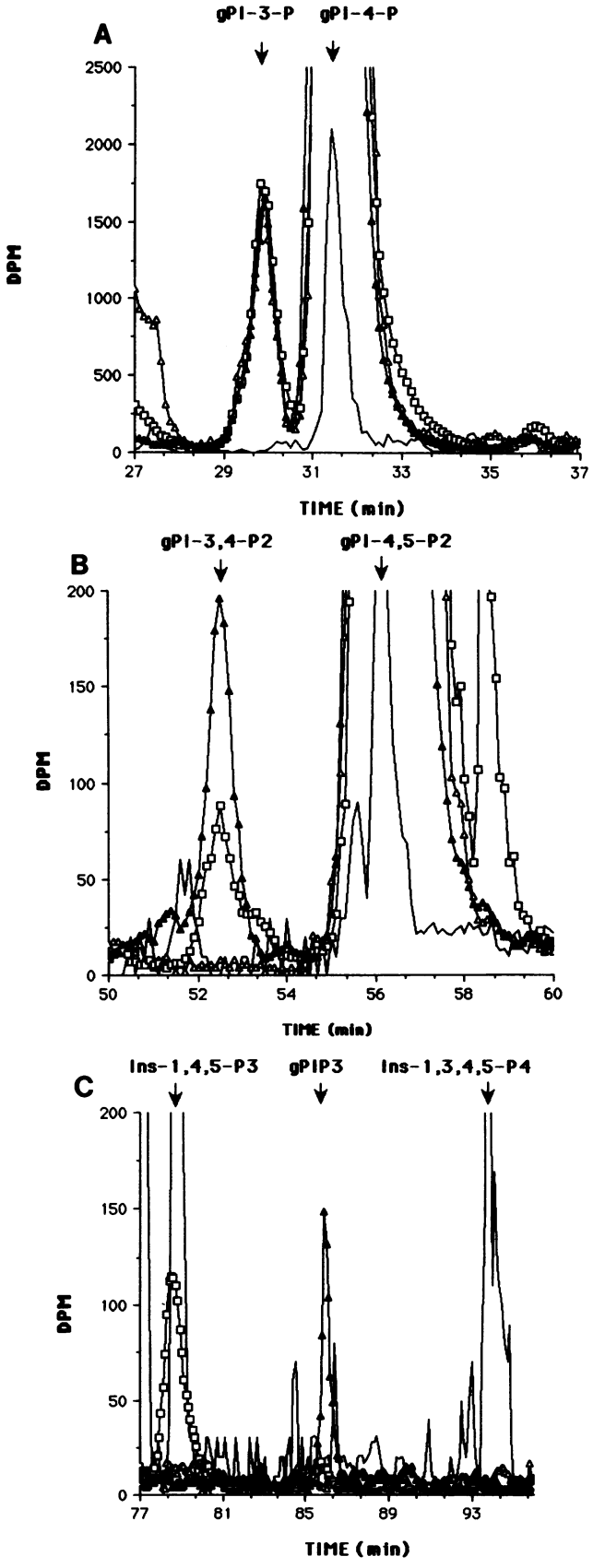

FIG. 4. HPLC identification of polyphosphoinositides in intact cells. Control NIH 3T3 cells and cells expressing BCR/*abl* and transforming *gag*/BCR/*abl* proteins were labeled with 0.1 mCi of $^{32}PO_4^{3-}$ per ml for 3 h prior to harvest. At the end of the labeling period, cells were rinsed in serum-free medium, 1 ml of 1 N HCl was added to each plate, and total phospholipids were extracted into chloroform-methanol (2:1), dried under N_2, deacylated, and separated by HPLC as described in Materials and Methods. (A) HPLC tracing corresponding to gPI-3-P and gPI-4-P; (B) HPLC tracing corresponding to gPI-3,4-P_2 and gPI-4,5-P_2; (C) HPLC tracing corresponding to IP_3, PIP_3, and IP_4. Symbols: △, ^{32}P incorporated into deacylated phospholipids extracted from control NIH 3T3 cells; □, deacylated phospholipids from cells expressing BCR/*abl* protein; ▲, deacylated phospholipids from cells expressing *gag*/BCR/*abl* protein; ———, ^{3}H standards (NEN) coinjected with the ^{32}P-labeled cell extracts. The data from each experiment were normalized to 0.2 × 10^6 total cpm. The actual total counts per minute were as follows: NIH 3T3 cells, 89,300 dpm; BCR/*abl* protein-expressing cells, 131,500 dpm; *gag*/BCR/*abl* protein-expressing cells, 121,200 dpm (in PI-4-P plus PI-4,5-P_2). The ratio of ^{32}P incorporated (PI/PI-4-P/PI-4,5-P_2) was not significantly different in each of the experiments. In some experiments a small amount of I-1,4,5-P_3 appears in the deacylated lipids (C) due to hydrolysis of PI-4,5-P_2. The peak following gPI-4,5-P_2 in panel B (59 min) has not been identified.

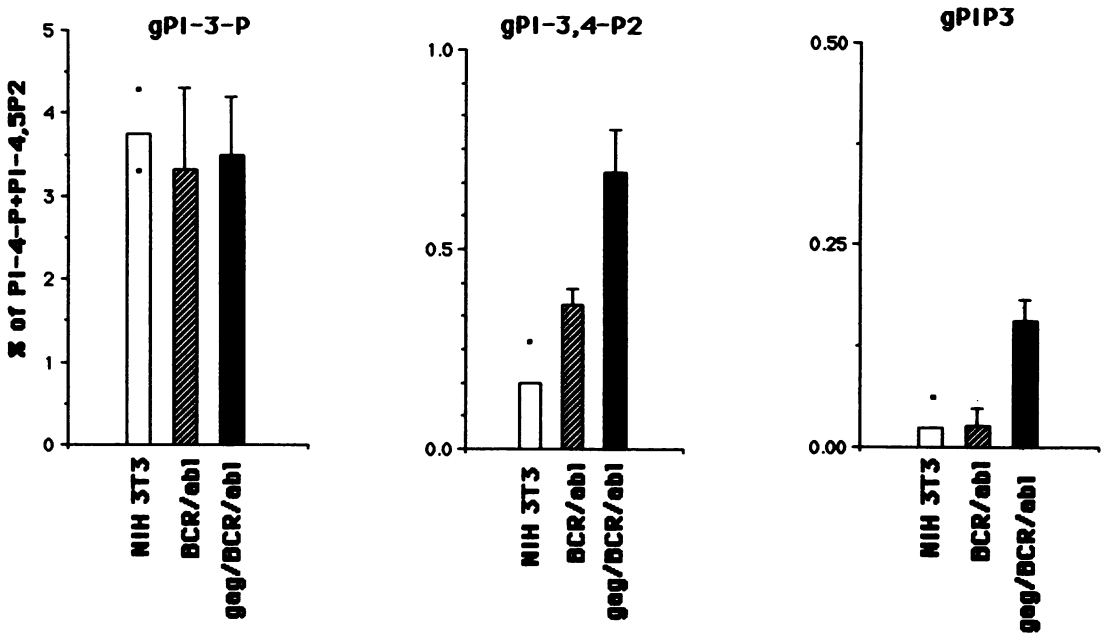

FIG. 5. Total ^{32}P incorporated into D-3-phosphorylated phosphoinositides in control and transfected NIH 3T3 cells. The total ^{32}P incorporated into D-3-phosphorylated polyphosphoinositides is expressed as the percentage of $^{32}PO_4^{3-}$ incorporated into PI-4-P plus PI-4,5-P_2. Symbols: □, results obtained from control NIH 3T3 cells (average of two experiments; the individual results are represented by black dots); ▨, results from cells expressing BCR/*abl* protein (three experiments; error bars are the standard deviations); ■, results from cells expressing *gag*/BCR/*abl* protein (four experiments; error bars are the standard deviations).

cells even when expressed at high levels (16; Fig. 6). We have shown that tyrosine-kinase-active forms of the *abl* protein associate with PI 3-kinase. An 85-kDa protein with a migration position similar to that of the 85-kDa subunit of PI 3-kinase was phosphorylated in vitro in anti-*abl* protein immunoprecipitates from ΔxB and *gag*/BCR/*abl* protein-transformed cells.

The inability of the normal c-*abl* type IV protein and the ΔxB kinase-deficient mutant protein (ΔxB-K290M) to associate with PI 3-kinase suggests that protein-tyrosine kinase

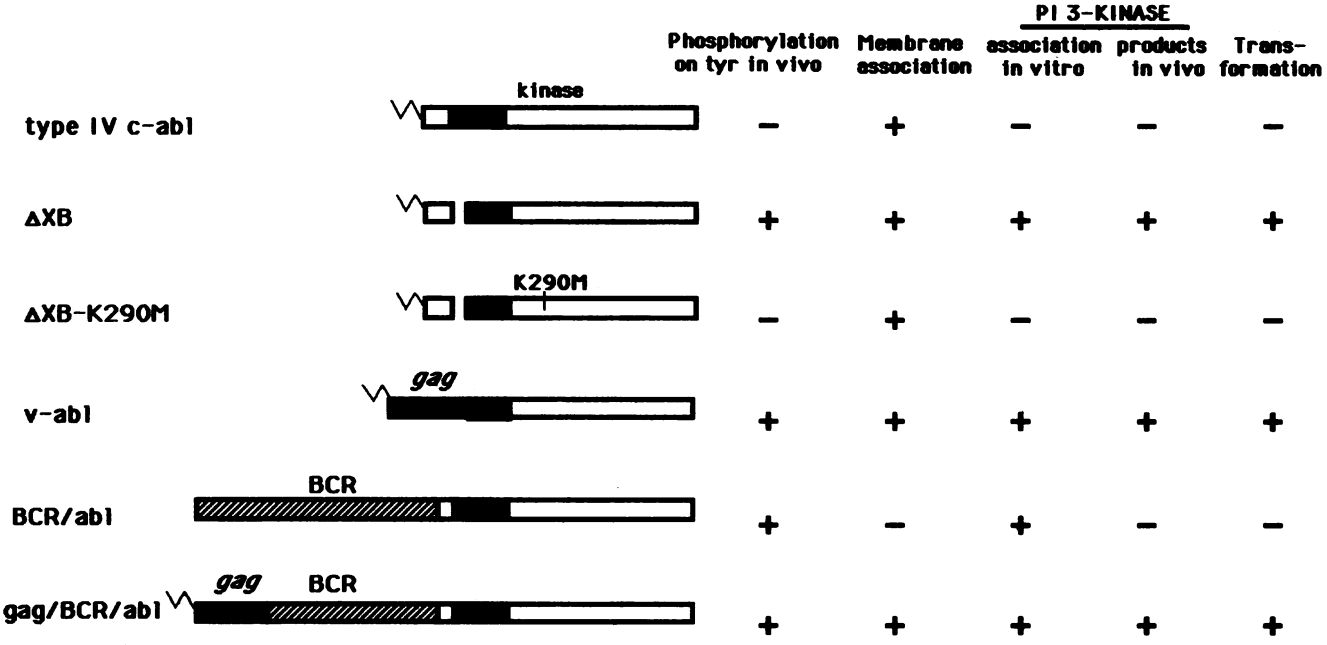

FIG. 6. Characteristics of the *abl* oncogene variants. SH domains represent the *src* homology regions. Symbols: ⋀, myristoyl fatty acid; ▨, SH 3; ▤, SH 2; ■, *gag* sequence derived from helper virus.

activity is necessary for association of *abl* proteins with PI 3-kinase and implicates PI 3-kinase as an important target in transformation.

In addition to an activated protein-tyrosine kinase, the *abl* protein appears to need amino (N)-terminal myristoylation for efficient transformation of fibroblasts. The v-*abl* oncogene product and Δ×B mutant protein have the myristoylation sequence and efficiently transform cells. The BCR/*abl* protein lacks the myristoylation sequence and fails to transform NIH 3T3 cells, although it does confer growth factor independency for some lymphocyte cell lines (8, 26) and weakly transforms Rat-1 cells (23). Attachment of the retrovirus *gag* protein sequence to the N terminus of the BCR/*abl* protein provides a myristoylation sequence and makes this gene product strongly transforming for NIH 3T3 fibroblasts (9). The importance of amino-terminal myristoylation is also supported by results obtained with v-*abl* and Δ×B gene products which lack the myristoylation sequence and fail to transform fibroblasts (16a).

The characteristics of the *abl* protein variants which were studied are summarized in Fig. 6. Mutants in which the *abl* protein failed to activate PI 3-kinase had decreased levels of PI-3,4-P_2 and extremely low levels of PIP_3 and were transformation defective. The BCR/*abl* protein has high protein-tyrosine kinase activity and associates with PI 3-kinase but is not transforming. Significantly, cells transfected with the BCR/*abl* gene did not have detectable PIP_3. These results suggest that mere association of the PI 3-kinase with an activated protein-tyrosine kinase is insufficient to increase the level of products of the enzyme in intact cells. The implication is that amino-terminal myristoylation is also needed to place the *abl* protein/PI 3-kinase complex at a membrane location where lipid phosphorylation can occur efficiently. Thus, transformation by the *abl* oncogene product correlates better with the cellular levels of PIP_3 than with *abl*-associated PI 3-kinase activity. An analogous $pp60^{v\text{-}src}$ mutant protein, which lacks the amino-terminal myristoylation sequence, was also found to associate with PI 3-kinase (11) but not to transform fibroblasts (17). However, the levels of PI-3,4-P_2 and PIP_3 in vivo were not investigated. The present results predict that PIP_3 would not be present under quiescent conditions in those cells.

The role of D-3-phosphorylated polyphosphoinositides in signal transduction is unclear. The pathways for synthesizing and degrading these lipids are complicated and appear to be regulated at multiple steps by different growth factors and hormones (3). As a consequence, the cellular ratios of PI-3-P to PI-3,4-P_2 to PIP_3 vary dramatically in response to growth factors, hormones, and transformation by oncogenes. Although PI-3,4-P_2 and PIP_3 can be produced by phosphorylation of PI-4-P and PI-4,5-P_2, respectively, by a purified PI 3-kinase (4), an alternative pathway for PI-3,4-P_2 synthesis exists, involving phosphorylation of PI-3-P at the D-4 position (45). None of the D-3-phosphorylated lipids is a substrate for the PI-specific phospholipases C that have been investigated (22, 37), indicating that the appearance of these lipids is controlled independently of the canonical PI turnover pathway. Thus far, the evidence that these lipids regulate growth is based on mutational studies of growth factor receptors and oncogenes. Further biochemical studies to determine their cellular targets are under way.

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